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Sewage sludge as a soil amendment in a *Larix decidua* plantation: Effects on tree growth and floristic diversity

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HIGHLIGHTS

- The impacts of sewage sludge application in larch plantation were studied.
- Lack of sewage sludge effects on soil and underwood diversity.
- No significant effects on diameter and height growth of larch tree
- No accumulation of Cu, Zn, Pb and Cd in larch needles and edible sporocarps
- Small dose (0.4 t DW ha^{-1}) explains lack of effects on analyzed parameters

GRAPHICAL ABSTRACT

Sludge application
at 0.4 t ha-1
(June 2008)
Sludge effects on:
- the two horizons: litter and underlying soil
- diameter and height growth of larch tree
- floristic communities in the underwood
- trace metal transfer in soil-larch-mushroom continuum
"Small dose explains lack of effects
concerning analyzed parameters"

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ABSTRACT

Sewage sludge application in forest plantations is an interesting complementary alternative practice to sewage sludge reutilization and recycling, with a significant and sustainable net effect in climate change mitigation. However, to optimize it a detailed knowledge of its effects on ecosystem components such as plants, soil, water and fauna is needed. We investigated the effects of sewage sludge application on soil, tree growth and floristic diversity in a ten-year-old plantation of European larch (*Larix decidua* Mill.). Our one-hectare study site, located at Mélisey, Haute-Saône, France (47°753′ Lat., 6°580′ Long.), was subdivided into six plots. Three plots, alternating with three control plots (no sewage sludge application), were amended in June 2008 with 0.4 t DW ha^{-1} obtained from a municipal urban wastewater treatment plant in Mélisey. Within each plot, one subplot was delimited and sludge was again manually applied at 3 t of DW ha^{-1} in July 2009 and March 2010 to the soil surface of the amended subplots without incorporation. The results showed no effect on radial and height growth of European larch amended with 0.4 t DW ha^{-1}. While a significant temporary increase in pH, macro-element contents (N, P and Ca) and the trace metal (Cu and Zn) concentration in the soil was observed, it had no significant effect on needles and sporocarp contents. The number of species in the amended subplots with 3 t DW ha^{-1} $year^{-1}$ increased by 80% compared to the control. However, the relative species abundance present only in amended subplots remains <1, except for *Hypericum humifusum*.

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1. Introduction

Sustainable water quality management is a major concern in today's context of climate change. Rising temperatures have led to extensive wastewater treatment in order to produce water of suitable quality to be returned to the receiver environment with minimal impact on the ecosystem or on public health. These treatments also generate sewage sludge (SS), a complex sub-product that has been extensively spread over agricultural lands as fertilizer (Singh and Agrawal, 2008). In France, for example, 73% of municipal sludge is currently considered to be of value in agriculture (Legroux and Truchot, 2009). Recently,

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however, farmers have shown reluctance towards sludge as they have been heavily influenced by a negative public opinion about certain pollutants, emanations of unpleasant odors and real concerns about health-related risks (Bourioug et al., 2015a).

A recent rise in the potential demand for energy biomass production has resulted in an increased interest in forest plantation. However, though forest growth and yield depend on soil fertility, tree crop rotations may cause soil nutrient depletion (Jama and Nowak, 2012). Thus, SS application in forest plantations is an interesting alternative practice to SS reutilization and recycling, with a significant and sustainable net effect on climate change mitigation. In addition, it is assumed, due to the small percentage of forest products found in the human diet (Przewrocki et al., 2004), that health endangerment as a consequence of pollutant transfer is lower than from agriculture generally.

Certain soils are well suited to woodland development, and are therefore subject to practices such as coppicing to produce biomass for energy production purposes and wood products. In these plantations, the spreading of sludge on the forest floor can improve soil characteristics by, for example, providing macro- and microelements, improving soil organic matter, increasing water retention and pH, etc. (Bailly et al., 2004; Ferrer et al., 2011; Bourioug et al., 2015b) and thus enhance tree growth (Saruhan et al., 2010). In addition, sludge encourages soil aeration by stimulating root growth and increasing worm and micro-organism populations (Singh and Agrawal, 2008; Lyberatos et al., 2011). The net effect is, thus, improved soil quality and greater biomass yields.

However, there are various types of contaminants present in SS such as trace metals, organic pollutants, medically active substances, etc. Sludge spreading therefore requires intensive monitoring of ecosystem components such as plants, soil, water and fauna to identify any possible positive or negative impacts on plant productivity, on the environment and on human health (Carnus and Thomas-Chery, 2007; Bourioug et al., 2015a).

The objective of this study was to determine the effects of SS application on the soil, and on the diameter and height growth, and on floristic diversity in a ten-year-old plantation of European larch (*Larix decidua* Mill.).

2. Materials and methods

2.1. Experiment site and sewage sludge properties

The experiment was carried out in a 10-year-old coniferous *Larix decidua* plantation with inter- and intra-row spacing of 3.5 and 2.5 m respectively, located at Mélisey, Haute-Saône, France (47°753′ Lat., 6°580′ Long.) (Fig. 1). The climate is semi-continental with the highest monthly temperature at 27.8 °C in July and the lowest at −3.7 °C in January. Annual precipitation is 662 mm (data 2010) (Linternaute.com, 2004). The soil is a clay loam with 18% clay, 33.7% loam and 48.3% sand and classified as a pseudo luvisol with dysmull. The study site, located at an altitude of 384 m, measured 100 m × 105 m with a slope of about 10% and was subdivided into six plots (17.5 m × 100 m). Three of the six plots (A1, A2 and A3) were amended in June 2008 with 0.4 t DW ha^{-1} (tons dry weight per hectare) of SS obtained from a municipal urban wastewater treatment plant in Mélisey. Three alternating control plots (C1, C2 and C3) received no sewage sludge application. Within each plot, one 2.5 × 2.5 m subplot was delimited randomly (A′1, A′2, A′3, C′1, C′2 and C′3) (Fig. 2). SS was again, in July 2009 and March 2010, manually applied to the soil surface of the amended subplots without incorporation, 3 t DW ha^{-1} each time. The physico-chemical characteristics of the soil and SS are provided in Table 1.

2.2. Sampling

For each subplot, the soil was sampled at both litter level (0 to 5 cm depth) and in the underlying soil (5 to 20 cm depth). At each depth, we

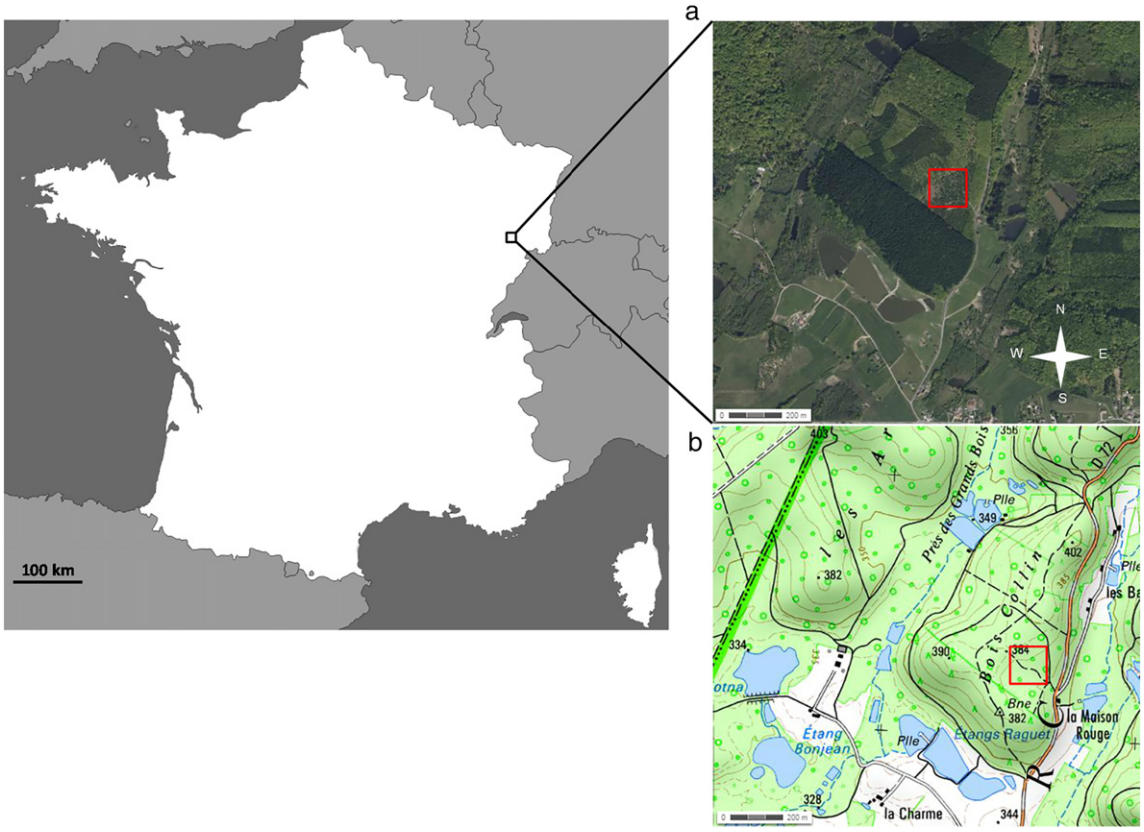

Fig. 1. Geographic location study site (Mélisey, Haute-Saône, France (47°753′ Lat., 6°580′ Long.)). a: Aerial photo, b: Topographic map (Adapted from Geoportail France, 2015).

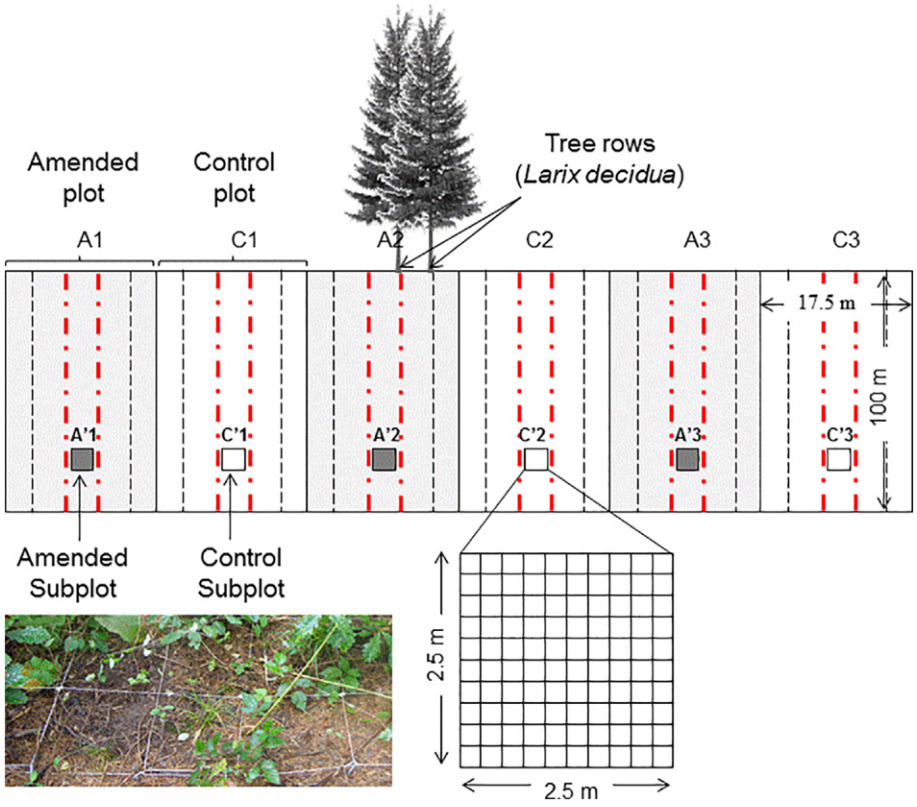

Fig. 2. Schematic representation of study area.

collected three samples on each subplot which were then mixed together to obtain a composite sample. Samples were collected monthly from March 2010, before the second sewage sludge application to the subplots, to August 2010. The needle samples, pooled from the four trees bordering each subplot, were taken in June 2010 from each of the 6 subplots. Mushrooms were collected in autumn 2010 in each plot. The samples (needles and sporocarps) were transported in an ice box to the laboratory, where, they were stored at −80 °C. Soil samples were air-dried at 30 °C until constant weight, and were then ground and sieved (2 mm mesh), while needle and sporocarp samples were lyophilized using freeze-drying technique and ground into a fine powder.

Table 1
Characteristics of soil and sewage sludge used in the experiment.

Parameters (unit)	Soil (mean ± SD)	SS (mean ± SD)	Soil limits[a]	SS limits[a]
Cu (μg g^{-1} DW)	12.1 ± 0.9	876.7 ± 39.6	100	1000
Zn (μg g^{-1} DW)	29.6 ± 3.11	429.5 ± 22.9	300	3000
Pb (μg g^{-1} DW)	24.9 ± 7.5	20.1 ± 2.7	100	800
Cd (μg g^{-1} DW)	0.06 ± 0.01	1.8 ± 0.1	2	10
Ca (mg g^{-1} DW)	0.19 ± 0.07	13.3 ± 1.7		
Mg (mg g^{-1} DW)	0.02 ± 0.01	3.7 ± 0.1		
Fe (mg g^{-1} DW)	10.9 ± 1.4	5.2 ± 1.3		
Al (mg g^{-1} DW)	22.2 ± 0.4	5.7 ± 0.7		
N total (%)	0.16 ± 0.03	7.2 ± 0.6		
C total (%)	2.1 ± 0.2	41.6 ± 3.5		
P total (mg g^{-1} DW)	1.1 ± 0.2	15.5 ± 0.2		
P_{Olsen} (mg g^{-1} DW)	0.02 ± 0.008	3.9 ± 0.4		
K (mg g^{-1} DW)	1.6 ± 0.3	5.4 ± 1.1		
pH_{water} (2:5)	4.8 ± 0.2	5.7 ± 0.3		
OM total %	6.4 ± 0.7	83.5 ± 7.4		
Ratio C:N	13.1 ± 1.9	5.78 ± 0.4		
CEC (%)	5.35 ± 1.48	–		
Clay (%)	18 ± 0.3	–		
Loam (%)	33.7 ± 0.4	–		
Sand (%)	48.3 ± 0.6	–		

SD: standard deviation, $n = 5$.
[a] French regulatory limits (08/01/98) for concentration of TMs in SS for agricultural use.

2.3. Analyses

Soil pH was determined using a pH-meter (WTW pH 300i Meter). Measurements were taken with a soil/water ratio of 2:5 (w:v) shacked for 2 h (Fuentes et al., 2007). Ammonium and nitrate were extracted with 2 M KCl using a 1:10 soil-extractant ratio and a 1 h end-over-end shake followed by filtration (Blakemore et al., 1987) and stored at −20 °C until analysis. The ammonium content was determined by the colorimetric method NFT 90-015-1 and nitrate content by ionic chromatography according to the standard method ISO 10304-1. Kjeldahl nitrogen (N_k) levels were determined by the Kjeldahl method (Mathieu and Pieltain, 2003). Soil, needle and sporocarp samples were digested by ISO 11466 (1995) and by the method described by García-Delgado et al. (2007). The mineral element (P, K, Mn, Mg and Ca) and trace metal (Zn, Cu, Pb and Cd) concentrations were determined using inductively coupled plasma mass spectroscopy (ICP-MS). All analyses were carried out in triplicate. The obtained values agree well (within 5%) with the certified concentrations in the standard references (calcareous loam soil n°141R from BCR in Belgium, and Virginia tobacco leaves "CTA-VTL-2" in Poland), confirming the reliability of our analytical procedure.

2.4. Measured tree parameters

Larch growth in height and girth was monitored on two central rows of each plot (Dashed red line on the site diagram – Fig. 2). Dendrometric surveys were then carried out in 2011 by measuring with a flexible tape the circumference at breast height (1.30 m) and total height using the

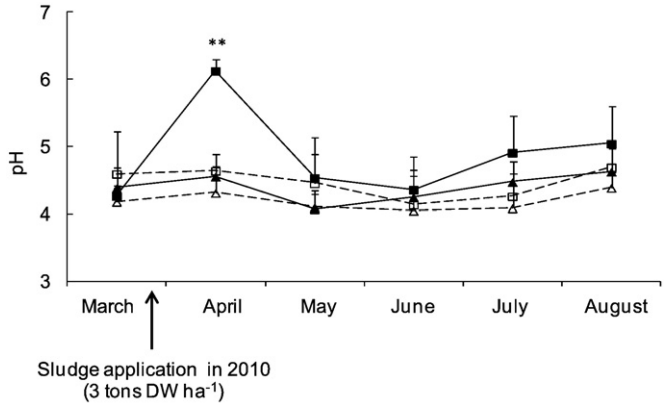

Fig. 3. pH changes in litter (solid line) and underlying soil (dotted line) of both control (triangles) and amended subplots (square symbols) with two sewage sludge applications of 3 t DW ha^{-1} (July 2009 and March 2010). Measurements were taken after second application from March to August. Results are means ± SD (n = 3). Significance is indicated by asterisks (** for p-value <0.01).

optical height meter (clinometer). These measurements were made in late autumn when diameter and height growth had ceased. In order to accurately characterize tree growth, the basal area (BA) was calculated for each tree using the formula (1):

$$BA = \frac{C130^2}{4\pi} \quad (1)$$

where C130: girth measured at 1.30 m above soil surface.

2.5. Floristic communities in the underwood

Undergrowth species diversity will provide an indication of the potential SS impact on biodiversity. SS can modify physical and chemical properties of soil, which, in turn, can influence the flora present on the amended site.

In May 2008, 2009 and 2010, inventories of the flora were carried out in the C and A plots. For each of the species recorded, an abundance index was attributed (0 = species represented by <10 individuals in the plot; 1 = species covering <5% of the herbaceous stratum; 2 = from 5 to 25%, 3 = from 26 to 50%, 4 = from 51 to 75% and finally 5 = >75%). The widely used Sorensen similarity index (Magurran, 2004) measures similarity in species composition for two sites, A and B, with the Eq. (2):

$$Cs = \frac{2ab}{a+b} \quad (2)$$

where a is the number of species found at site A; b is the number of species at site B and ab is the number of species shared by the two sites.

In May 2010, a supplementary floristic survey was carried out in the subplots divided to quadrants of 25 × 25 cm (Fig. 2). Thus, the number of individuals of each species were determined in each subplot to calculate the relative abundance of each species (RA%).

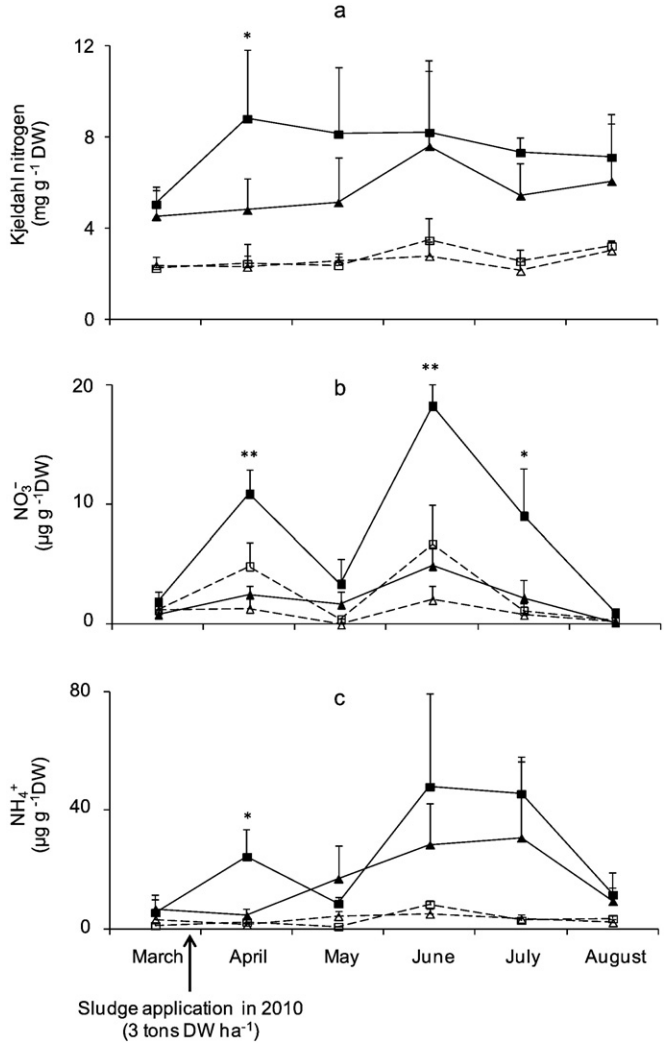

Fig. 4. Changes in Kjeldahl nitrogen (mg g^{-1} DW) (a), nitrates (µg g^{-1} DW) (b) and ammonium (µg g^{-1} DW) (c) in litter (solid line) and underlying soil (dotted line) of both control (triangles) and amended subplots (squares) with two sewage sludge applications of 3 t DW ha^{-1} (July 2009 and March 2010). Measurements were taken after second application from March to August. Results are means ± SD (n = 3). Significance is indicated by asterisks (*p < 0.05 and **p < 0.01).

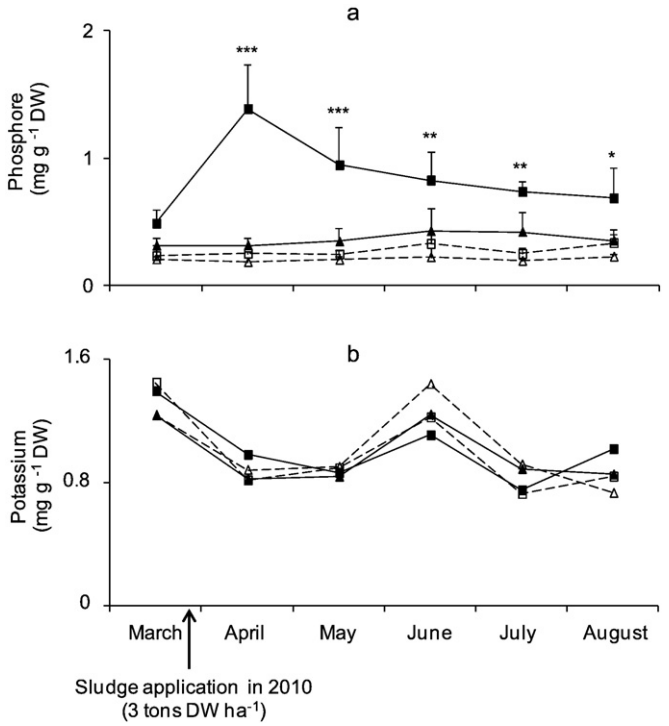

Fig. 5. Phosphore (mg g^{-1} DW) (a) and potassium (mg g^{-1} DW) (b) changes in litter (solid line) and underlying soil (dotted line) of both control (triangles) and amended subplots (square symbols) with two sewage sludge applications of 3 t DW ha^{-1} (July 2009 and March 2010). Measurements were taken after second application from March to August. Results are means ± SD (n = 3). Significance is indicated by asterisks (*p < 0.05, **p < 0.01 and ***p < 0.001).

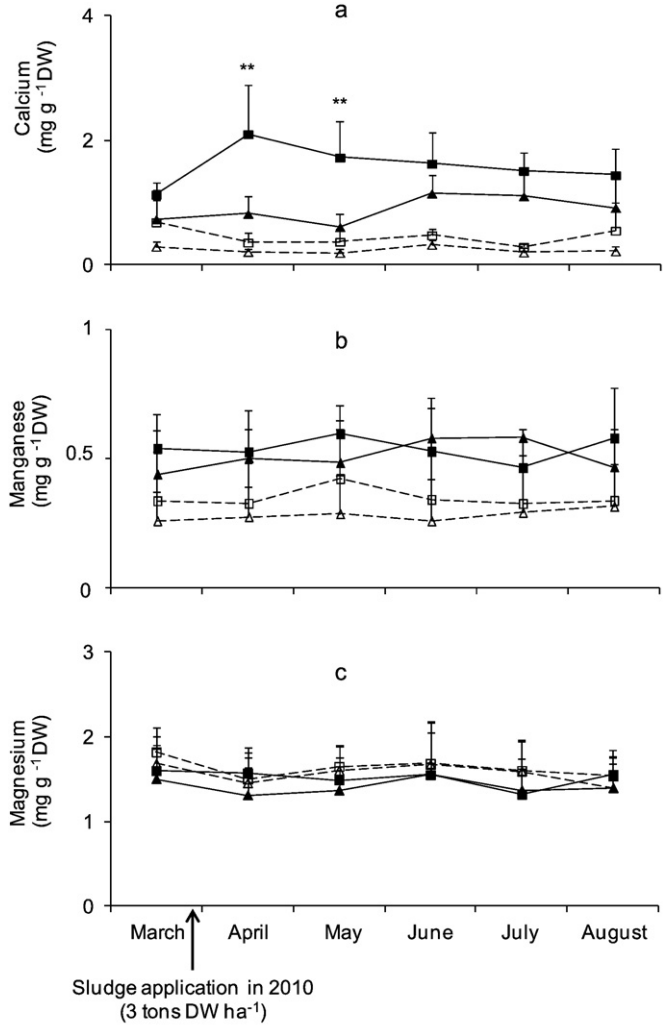

Fig. 6. Concentration changes (mg g^{-1} DW) in calcium (a), manganese (b) and magnesium in litter (solid line) and underlying soil (dotted line) of both control (triangles) and amended subplots (squares) with two sewage sludge applications of 3 t DW ha^{-1} (July 2009 and March 2010). Measurements were taken after second application from March to August. Results are means ± SD ($n = 3$). Significance is indicated by asterisks ($^{**}p < 0.01$).

2.6. Statistical analysis

Statistical analyses were performed with the Graphpad Prism 5 software (GraphPad Software, Inc.). Data were first tested for normality and homogeneity of variance to check statistical demands. The data were then analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A p value of <5% ($p < 0.05$) was considered statistically significant.

3. Results

3.1. SS effects on soil

3.1.1. pH

The average pH value of the two horizons: litter and underlying soil before sludge was 4.5. The addition of 3 t DW ha^{-1} of liquid sludge in March 2010 resulted in a significant increase in litter pH (1.5 units). Nevertheless, it was only temporary because two months after SS application, the pH dropped to its initial value (Fig. 3). The pH of the soil underlying the horizon of the amended subplots varies little and remains similar to both horizons in control subplots.

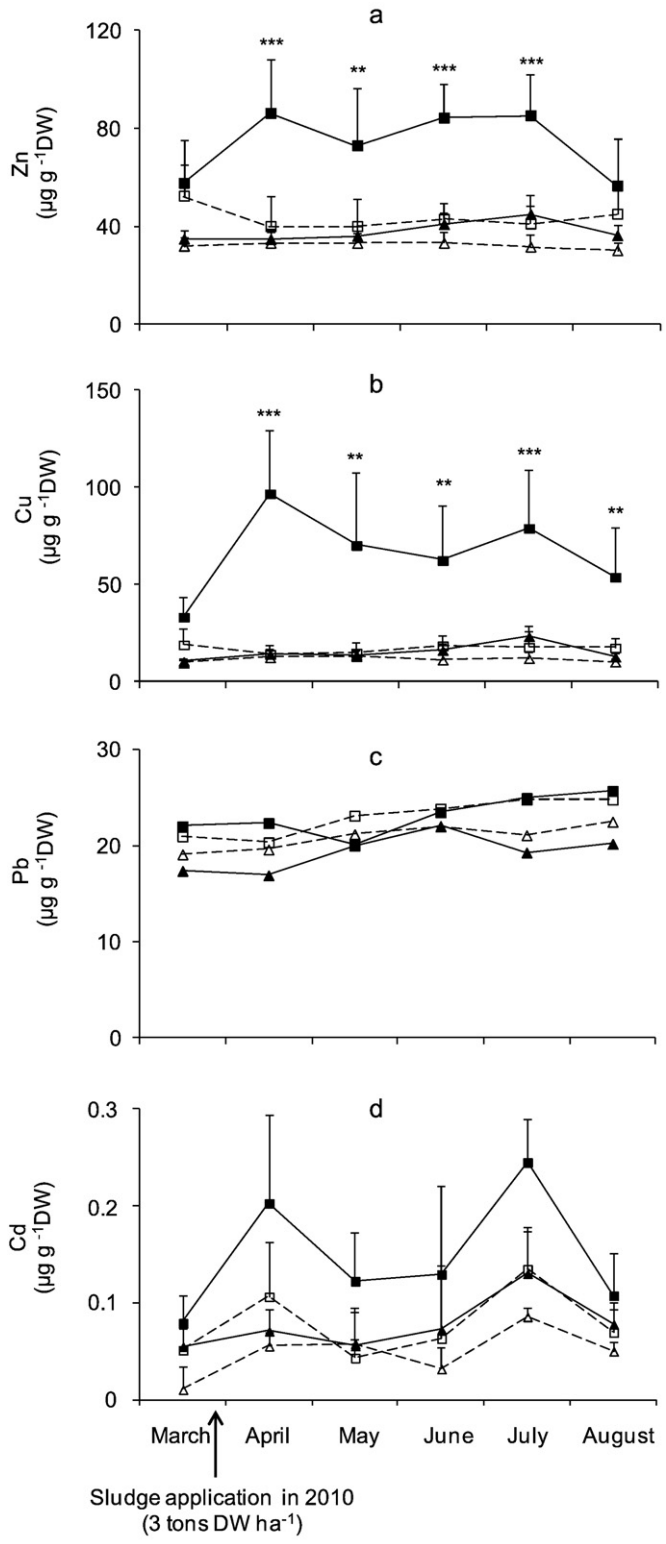

Fig. 7. Changes in trace metal concentrations (µg g^{-1} DW) (zinc (a), copper (b), lead (c) and cadmium (d)) in litter (solid line) and underlying soil (dotted line) of both control (triangles) and amended subplots (squares) with two sewage sludge applications of 3 t DW ha^{-1} (July 2009 and March 2010). Measurements were taken after second application, from March to August. Results are means ± SD ($n = 3$). Significance is indicated by asterisks ($^{**}p < 0.01$ and $^{***}p < 0.001$).

3.1.2. N, P and K

Fig. 4 shows Kjeldahl nitrogen Nk (a), nitrates NO_3^- (b) and ammonium NH_4^+ (c) concentrations in litter and underlying soil. One month

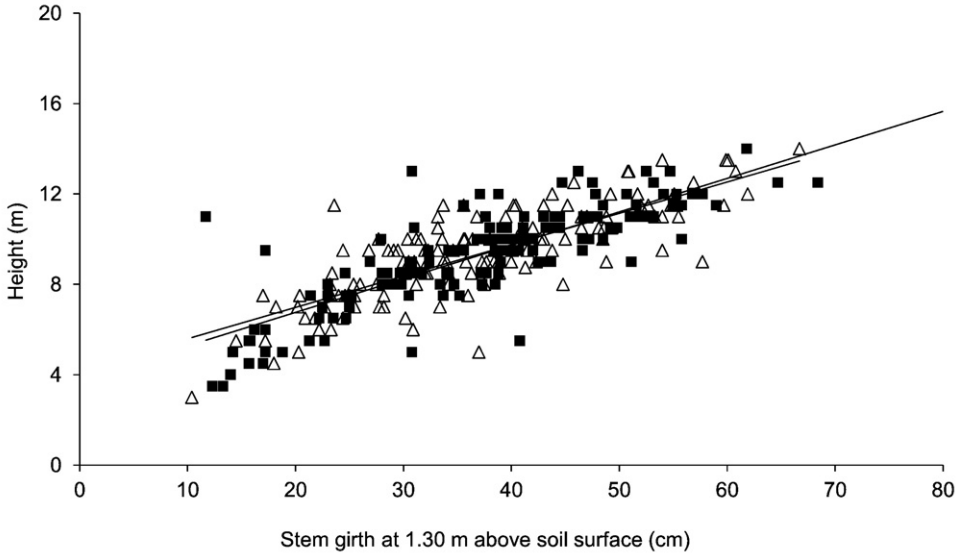

Fig. 8. Relationship between stem girth at 1.30 m above soil surface and larch tree height in control (△) and amended plots (■) throughout the study site (sewage sludge applied at 0.4 t DW ha^{-1} in June 2008).

after the second application, Nk content increased significantly (twice as high) in the litter of the amended subplots compared to control. It then decreased gradually, but between treatments no significant difference was observed. In the litter of amended plots, nitrates and ammonium showed two significantly different peaks. The first was recorded in April (one month after SS application), when NO_3^- and NH_4^+ were five times higher than those measured in control litter. Despite the higher values obtained during the second peak, recorded in June, the nitrate and ammonium contents were, respectively, about 4 times and 2 times higher in the litter of the amended subplots compared to the control litter. For the underlying soil, the contents of different nitrogen forms showed no significant difference between control and amended treatment.

Compared to control, the P content increased significantly in the litter of the amended subplot (Fig. 5a). The maximum was 1.4 mg g^{-1} DW reached after SS application, gradually decreasing to 0.68 mg g^{-1} at the end of follow-up. In contrast, the sludge had no significant effect on the P content of the underlying soil, which was stable at around 0.2 mg g^{-1} DW, as for both horizons of the control modality. Regardless of the treatment, horizon and sampling time, the potassium contents were similar and showed the same trends (Fig. 5b). Means fluctuated from 0.73 to 1.45 mg g^{-1} DW.

3.1.3. Ca, Mg and Mn

After SS application, Ca content in the litter increased significantly compared to the control (Fig. 6a). It was 162 and 183% higher in April

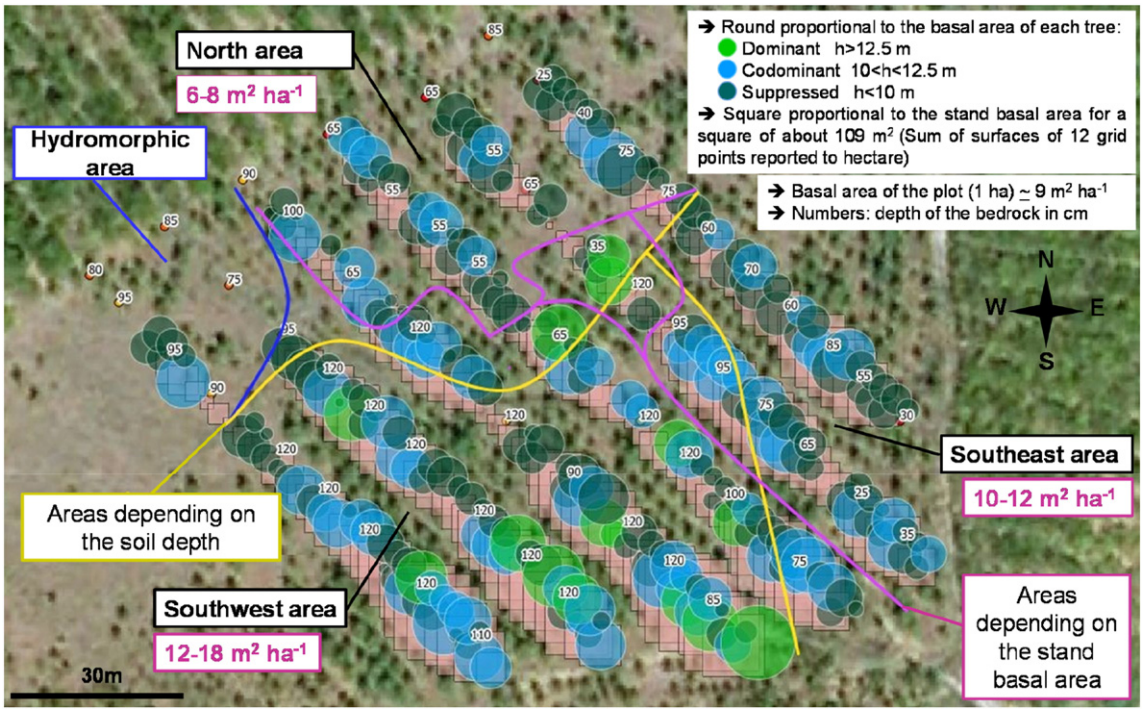
North area
6-8 m² ha⁻¹
Hydromorphic area
Areas depending on the soil depth
Southwest area
12-18 m² ha⁻¹
Southeast area
10-12 m² ha⁻¹
Areas depending on the stand basal area
30m
➔ Round proportional to the basal area of each tree:
Dominant h>12.5 m
Codominant 10<h<12.5 m
Suppressed h<10 m
➔ Square proportional to the stand basal area for a square of about 109 m² (Sum of surfaces of 12 grid points reported to hectare)
➔ Basal area of the plot (1 ha) ≃ 9 m² ha⁻¹
➔ Numbers: depth of the bedrock in cm
N
W
E
S

Fig. 9. Mapping homogeneous zones defined according to dendrometric and soil depth at the experiment site.

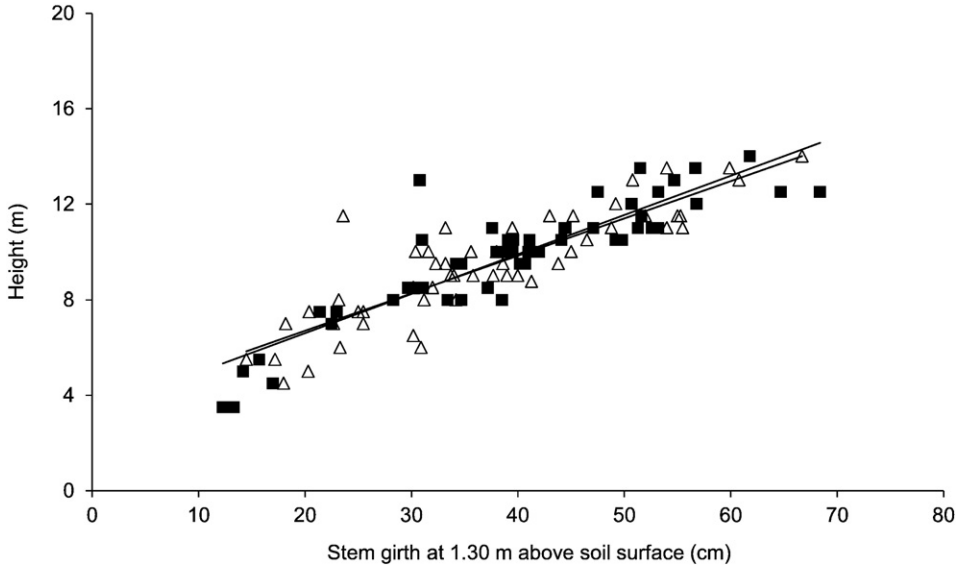

Fig. 10. Relationship between stem girth at 1.30 m above soil surface and larch tree height in control (△) and amended plots (■) within southwest zone (sewage sludge applied at 0.4 t DW ha^{-1} in June 2008).

and May, respectively, and then decreased progressively. In contrast, the measured values in the underlying soil did not show any significant difference between amended and unamended subplots. The SS does not affect soil Mg and Mn levels regardless of treatment, horizon or sampling time (Fig. 6b and c).

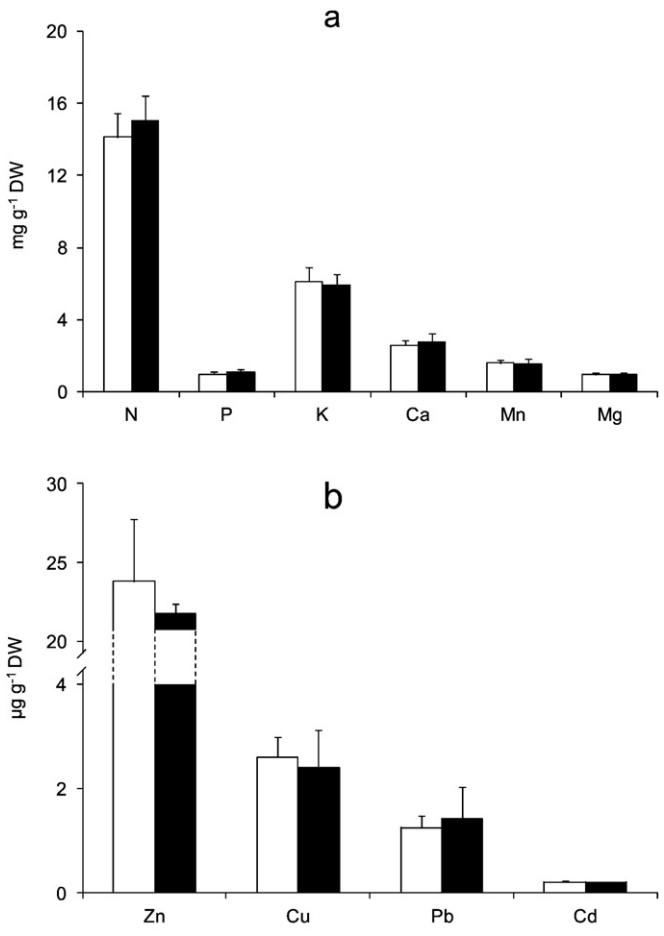

Fig. 11. Concentrations of mineral elements (N, P, K, Ca, Mn and Mg) in mg g^{-1} DW and trace metals (Zn, Cu, Pb and Cd) in µg g^{-1} DW in needle samples pooled from the four larch trees *L. decidua* surrounding each subplot (Control: white column and amended plots with 0.4 t DW ha^{-1}: black column).

3.1.4. Cu, Zn, Pb and Cd

SS application caused a significant increase in total zinc, copper and cadmium contents in litter horizon (Fig. 7). In April, the enrichment coefficients were 7.6, 2.6 and 2.8 for Cu, Zn and Cd, respectively. In the underlying soil horizons, no change occurred after sludge application and the trace metal contents studied remained stable and identical to those of control modality. SS application does not generate a significant variation in the Pb contents, whatever the horizon and the sampling time.

3.2. SS effects on larch trees

3.2.1. Radial and height growth

Fig. 8 shows that the relationship between stem girth and larch tree height was similar between amended (A) and control plots (C). In addition, great heterogeneity was observed within each modality.

Trees at the study site were characterized by their social status: Dominant (Do), Codominant (Co) and suppressed (Sup) (Fig. 9). This classification procedure corresponded to the stratification based on BA for each tree. Suppressed trees were the smallest trees in height (and consequently in diameter). Thus, three BA classes (in m^2 ha^{-1}) were identified: Low ([6–8]), Medium ([10 − 12]) and High ([12–18]), with an average of 9 m^2 ha^{-1}. Based on hydromorphy, soil depth and the BA distribution, the study site shows four homogeneous zones. In the southwest area, soil depths ranged from 85 to 120 cm. The BA and tree population are the largest, compared to the other zones. In this zone, study of radial and height growth only leads to the same results (Fig. 10).

3.2.2. Minerals and trace metal transfer to needles

The needles from the larch trees bordering the subplots were gathered in spring 2010. The results obtained for all the elements shows no difference between the amended and control subplots (Fig. 11).

3.3. SS effects on floristic communities in the underwood

Fig. 12 shows the specific richness changes according to the floristic surveys of 2008, 2009 and 2010 in the amended (A) and unamended (C) plots. The detailed results given in Tables 2, 3 and 4 concern the

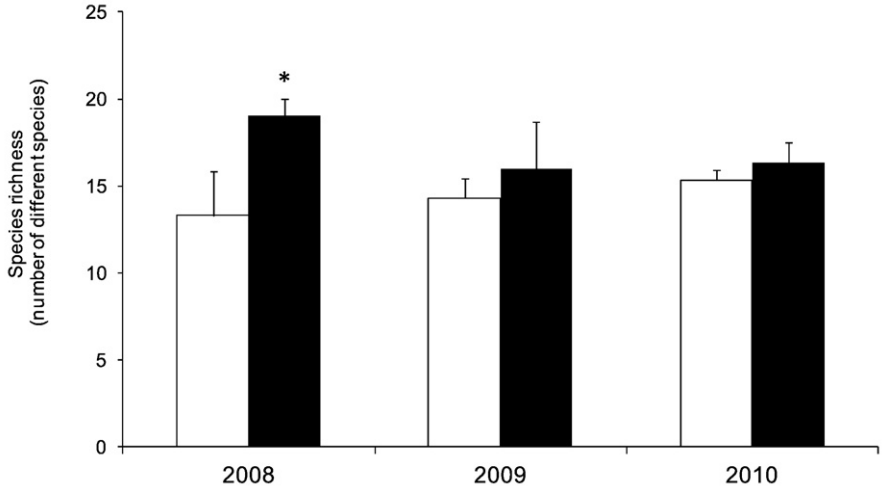

Fig. 12. Species richness changes in 2008, 2009 and 2010 in control (white column) and amended plots (black column) (sewage sludge of 0.4 t DW ha^{-1} in June 2008).

floristic surveys from the subplots. The most common species in the underwood are: common bent (*Agrostis capillaris*), wood bush (*Rubus fruticosus*), purple moor-grass (*Molinia caerulea*) and meadow vetchling (*Lathyrus pratensis*). The floristic survey conducted during the first year after SS application (2008) shows a significant increase in the specific richness of the amended plots (A) compared to the control (C), whereas in 2009 and 2010 no significant differences were observed. In addition, the Sorensen similarity index between amended (A) and control plots (C) is, respectively, 94.7, 88.2 and 97.1% in 2008, 2009 and 2010. The quantitative survey carried out in the amended (A′) and unamended subplots (C′) also enabled a more detailed floristic description (Table 5). After SS application (3 t DW ha^{-1} $year^{-1}$ in 2009 and 2010), the number of species in the amended subplots (A′) increases by 80% compared to the control (C′). However, the relative abundance of species present only in amended subplots remains <1, except for *Hypericum humifusum*.

3.4. SS effects on minerals and trace-element transfer to mushrooms

The results obtained for the minerals and trace elements analyzed in all the collected sporocarps in amended (A) and control (C) plots are depicted in Fig. 13. No significant differences according to treatment were observed.

4. Discussion

Amending soil with SS modifies the physico-chemical properties of soils. These modifications are related to the chemical composition of the sludge and especially to the organic matter content (Barzegar

Table 2
Detailed floristic survey conducted in May 2008 on amended (A) and control (C) plots. An abundance index was attributed (0 = species represented by <10 individuals in the plot; 1 = species covering <5% of the herbaceous stratum; 2 = between 5 and 25%, 3 = between 26 and 50%, 4 = between 51 and 75% and finally 5 ≥ 75%).

Species	Plots						Presence (1)/absence (0)		Similarity
	A1	C1	A2	C2	A3	C3	C	A	
Rubus fruticosus	3	4	4	5	3	4	1	1	1
Molinia caerulea	2	2	2	2	2	2	1	1	1
Hypericum humifusum and *H. pulchrum*	2	1	0	1	2	3	1	1	1
Eupatorium cannabinum	1	1	1	1	1	1	1	1	1
Salix caprea	1	0	1	0	1	0	0	1	0
Melampyrum pratense	2	1	0	1	2	3	1	1	1
Lathyrus pratensis	2	2	2	2	2	2	1	1	1
Agrostis capillaris	5	5	5	5	5	5	1	1	1
Lonicera periclymenum	2	1	0	1	2	3	1	1	1
Lycopersicon esculentum	1	0	0	1	2	0	1	1	1
Glechoma hederacea	1	1	1	1	1	1	1	1	1
Juncus effusus	1	1	1	1	1	1	1	1	1
Heracleum sp.	1	1	1	1	1	1	1	1	1
Carex pairae and *C. pilulifera*	1	1	1	1	2	0	1	1	1
Fraxinus excelsior	1	0	0	1	1	0	1	1	1
Crataegus sp.	1	1	1	1	1	0	1	1	1
Trifolium sp.	1	0	1	0	0	0	0	1	0
Poaceae sp. 1	5	4	3	2	1	0	1	1	1
Poaceae sp. 2	1	1	0	2	3	0	1	1	1
Poaceae sp. 3	1	1	0	2	3	0	1	1	1

Table 3
Detailed floristic survey conducted in May 2009 on amended (A) and control (C) plots. An abundance index was attributed (0 = species represented by <10 individuals in the plot; 1 = species covering <5% of the herbaceous stratum; 2 = between 5 and 25%, 3 = between 26 and 50%, 4 = between 51 and 75% and finally 5 = >75%).

Species	Plots						Presence (1)/absence (0)		Similarity
	A1	C1	A2	C2	A3	C3	C	A	
Rubus fruticosus	3	4	4	5	3	4	1	1	1
Molinia caerulea	2	2	2	2	2	2	1	1	1
Hypericum humifusum and *H. pulchrum*	2	1	0	1	2	3	1	1	1
Eupatorium cannabinum	1	1	1	1	1	1	1	1	1
Salix caprea	1	0	1	0	1	0	0	1	0
Melampyrum pratense	2	1	0	1	2	3	1	1	1
Lathyrus pratensis	2	2	2	2	2	2	1	1	1
Agrostis capillaris	5	5	5	5	5	5	1	1	1
Lonicera periclymenum	2	1	0	1	2	3	1	1	1
Lycopersicon esculentum	–	–	–	–	–	–	–	–	–
Glechoma hederacea	3	4	5	6	7	8	1	1	1
Juncus effusus	1	1	1	1	1	1	1	1	1
Heracleum sp.	1	1	1	1	1	1	1	1	1
Carex pairae and *C. pilulifera*	1	1	2	0	0	0	1	1	1
Fraxinus excelsior	1	1	1	0	0	0	1	1	1
Crataegus sp.	1	1	1	0	0	0	1	1	1
Trifolium sp.	1	0	0	0	0	0	0	1	0
Poaceae sp. 1	5	4	3	2	1	0	1	1	1
Poaceae sp. 2	1	0	1	2	3	4	1	1	1
Poaceae sp. 3	1	0	1	2	3	4	1	1	1

Table 4
Detailed floristic survey conducted in May 2010 on amended (A) and control (C) plots. An abundance index was attributed (0 = species represented by <10 individuals in the plot; 1 = species covering <5% of the herbaceous stratum; 2 = between 5 and 25%, 3 = between 26 and 50%, 4 = between 51 and 75% and finally 5 = >75%).

Species	Plots						Presence (1)/absence (0)		Similarity
	A1	C1	A2	C2	A3	C3	C	A	
Rubus fruticosus	3	4	4	5	3	4	1	1	1
Molinia caerulea	2	2	2	2	2	2	1	1	1
Hypericum humifusum and *H. pulchrum*	2	1	0	1	2	3	1	1	1
Eupatorium cannabinum	1	1	1	1	1	1	1	1	1
Salix caprea	0	1	1	0	1	0	1	1	1
Melampyrum pratense	2	1	0	1	2	3	1	1	1
Lathyrus pratensis	2	2	2	2	2	2	1	1	1
Agrostis capillaris	3	3	4	4	3	4	1	1	1
Lonicera periclymenum	2	1	0	1	2	3	1	1	1
Lycopersicon esculentum	–	–	–	–	–	–	–	–	–
Glechoma hederacea	3	4	3	5	4	4	1	1	1
Juncus effusus	1	1	1	1	1	1	1	1	1
Heracleum sp.	1	1	1	1	1	1	1	1	1
Carex pairae and *C. pilulifera*	1	1	1	0	0	0	1	1	1
Fraxinus excelsior	–	–	–	–	–	–	–	–	–
Crataegus sp.	1	1	1	0	1	1	1	1	1
Trifolium sp.	1	1	1	1	1	1	1	1	1
Poaceae sp. 1	5	4	3	2	1	0	1	1	1
Poaceae sp. 2	1	0	1	2	3	4	1	1	1
Poaceae sp. 3	1	0	1	2	3	4	1	1	1

et al., 2002; Veeresh et al., 2003; Hussein, 2009; Kumar and Chopra, 2013; Belhaj Ayadi et al., 2016). Under our study conditions, the litter pH increases significantly after sludge application. After about two months, the pH level was again similar for the two treatments. This temporary increase is certainly due to the calcium concentration in the sludge (13.3 mg g^{-1}). Although low, its contribution made it possible to correct the pH values defined as optimal in agriculture (pH 6–7), thereby promoting microbial activity and increasing nutrient use efficiency by plants (Singh and Agrawal, 2008). SS is composed of organic and mineral matter, which concentrates on the topsoil, thus improving pH within the optimal value range. Similar results were obtained by

Table 5
Detailed floristic survey conducted in May 2010 on amended (A′) and control (C′) subplots based on numbers of species individuals. Relative Abundance (RA) = Species Absolute Abundance (SAA) / sum of all absolute abundances * 100.

Species	Number of individuals in each subplot						SAA A′	SAA C′	RA A′	RA C′
	A′1	C′1	A′2	C′2	A′3	C′3				
Agrostis capillaris	0	6	0	0	1	9	1	15	0.27	4.05
Betula pubescens	0	0	1	0	0	2	1	2	0.27	0.54
Carex pilulifera	19	7	31	1	11	7	61	15	16.49	4.05
Carpinus betulus	1	0	0	0	0	0	1	0	0.27	0
Deschampsia flexuosa	0	0	7	0	35	1	42	1	11.35	0.27
Digitalis purpurea	1	0	0	0	0	0	1	0	0.27	0
Frangula alnus	1	1	2	4	3	1	6	6	1.62	1.62
Galeopsis tetrahit	0	0	1	0	0	0	1	0	0.27	0
Holcus lanatus	0	0	1	0	0	0	1	0	0.27	0
Hypericum humifusum	3	0	0	0	1	0	4	0	1.08	0
Hypericum pulchrum	1	0	1	0	0	2	2	2	0.54	0.54
Juncus tenuis	1	0	0	0	0	0	1	0	0.27	0
Lonicera periclymenum	8	9	0	2	1	1	9	12	2.43	3.24
Luzula sylvatica	0	0	0	0	2	0	2	0	0.54	0
Pteridium aquilinum	18	8	5	6	19	4	42	18	11.35	4.86
Quercus robur	21	0	3	18	16	10	40	28	10.81	7.57
Rubus fruticosus	6	17	20	2	0	8	26	27	7.03	7.30
Spiraea ulmaria	0	0	0	0	3	0	3	0	0.81	0
Total	80	48	72	33	92	45	244	126	65.95	34.05

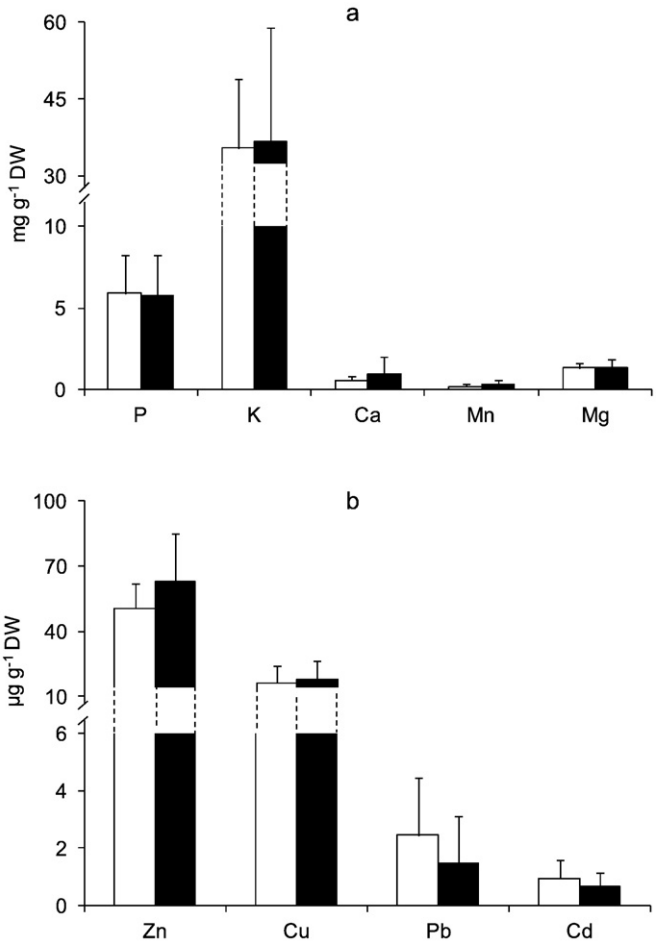

Fig. 13. Mineral element concentrations (P, K, Ca, Mn and Mg) in mg g^{-1} DW and trace metals (Zn, Cu, Pb and Cd) in µg g^{-1} DW in mushrooms collected from control (C) (white column) and amended plots (A) with 0.4 t DW ha^{-1} (black column).

Benbrahim et al. (2003), where applying composted sludge to podzolic soil (pH 4.5) resulted in an increase in pH to 5.7. Speciation of metals, complexation, adsorption and exchanges at the solid phase sites are closely related to soil pH (Cavallaro and McBride, 1984; Sauvé et al., 1997). Thus, acidic conditions favor the metal mobility and induce phyto-toxicity, while alkaline pH promotes the mineral element immobilization (Toribio and Romanya, 2006) and remains unavailable for plant absorption.

Concerning the soil's macro-element contents, our results reveal that only phosphorus increased significantly during monitoring period after SS application. Our results were similar to those obtained by Munir and Bayan (2004), Hussein (2009) and Belhaj Ayadi et al. (2016) who mentioned the high fertilizing ability of sludge for P nutrition. In the case of trace metals, the litter enrichment of amended subplots with Cu and Zn was related to initial high metallic content in SS. However, at the same study site and with the same experimental design, it has been demonstrated that the soil solution was not affected by these trace metal levels, thus avoiding possible groundwater contamination (Bourioug et al., 2015b).

Although sludge strongly stimulates growth, it is not always possible to demonstrate this beneficial effect, since ecosystems must have ecological and soil factor disparity. Dendro-metric measurements carried out on larch trees revealed wide heterogeneity. In addition, the relationship between height and circumference show that tree growth patterns don't vary with treatment. Soil depth plays a major role in influencing plant growth because greater soil depth provides a higher volume

available for the retention of nutrients and moisture (Myburgh et al., 1996; Matras and Pâques, 2008). At our study site, the BA and soil depth allowed us to define homogeneous areas. Thus, deeper soils show higher plant height, indicating the importance of soil depth to tree growth. Riou-Nivert (2001) showed that larch growth is optimal in deep and fertile soils because nutrient requirements are high in general.

Our results also show a sparse population located at the top of the study site. This zone is characterized by a very hydromorphic soil with a temporary water table at the 80 cm depth. Indeed, larch is one of the most sensitive tree species to root asphyxia (Bastien, 1997; Riou-Nivert, 2001), which explains the high seedling mortality observed. The advantage of this approach is to get rid of the influence of environmental factors on growth parameters and thus to identify a possible effect of SS application. Despite the definition of these homogeneous areas, no treatment effect was detected. This result may be attributed to the low dose applied (0.4 t DW ha^{-1}). Couillard and Grenier (1989) studied the effect of different SS doses on the *Larix laricina* growth and concluded that the effect absence is the result of one of the two factors: too small a dose or a very low mineralization rate when applying large doses. The SS dose thus clearly explains (0.4 t DW ha^{-1}) the lack of effect concerning the various parameters analyzed. However, the specific richness of the underwood increased significantly during the first year after SS application, but then returned to its initial state. Similar results were obtained by Carnus and Thomas-Chery (2007), but the specific richness decreases were observed only during the 3rd year after SS application and it is not a return to its original state but a change in the underwood flora. This phenomenon is well known in phytoecology with a species proliferation after fertilization or amendment, followed by a reduction in the specific richness due to the competition between species: some are more competitive and proliferate while others disappear.

Several saprobic fungi produce edible sporocarps commonly collected by humans. Due to mycelia ability to absorb trace metals from the environment, these sporocarps might accumulate these toxic elements and become unfit for human consumption. Thus, particular attention was paid to the sludge effects on the trace metal accumulation by fungi. Our results showed no significant effect, while Carnus and Thomas-Chery (2007) showed that liquid sludge significantly increased the Cu concentration in two species of fungi (*Hygrophoropsis aurantiaca* and *Xerocomus badius*), and that composted sludge and dehydrated lime sludge increased the Zn content in *Laccaria laccata* and *Mycena epipterygia*, respectively. These SS were applied in a dose of 3 t DW ha^{-1} $year^{-1}$. The results of these authors confirm that trace metal concentrations in sporocarps depend on the species and the considered element (Kalač and Svoboda, 2000).

5. Conclusion

The addition of sewage sludge to a ten-year-old plantation of European larch in very small doses (0.4 t ha^{-1}) clearly explains the lack of effect concerning tree growth and underwood diversity. Though the present data show some negative effects or no effects at all, this study will have to be completed by other experiments at various dose rates or repeated sewage sludge applications in order to evaluate the balance between beneficial effects and eventual toxicity attributable to high trace-metal concentrations in sludge.

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